

WHAT IS CLAIMED IS:

1. A method for establishing undifferentiated human embryonic stem cells, comprising the steps of:
 - (a) thawing a cryopreserved human blastocyst embryo; and
 - 5 (b) culturing at least a portion of said human blastocyst embryo on a medium capable of sustaining undifferentiated embryonic stem cells, whereby undifferentiated human embryonic stem cells are established.
2. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo comprises a sphere of
10 cells with an outer cell layer, a fluid filled cavity, and the inner cell mass.
3. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo comprises a human embryo that was cryopreserved from about 5 days to about 6 days after fertilization of said embryo.
- 15 4. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo has been cryogenically stored for more than four years.
5. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said thawing step comprises:
 - 20 (a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;
 - (b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and
25 cryoprotectant; wherein said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.

6. The method for establishing undifferentiated human embryonic stem cells of claim 5, wherein said cryoprotectant is selected from the group consisting of sucrose, glycerol and a combination of sucrose and glycerol.

7. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said thawing step consists of:

(a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;

(b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant;

(c) a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant;

(d) a subsequent fourth step of treating said cryopreserved human blastocyst embryo with a fourth solution comprising hFF and cryoprotectant; wherein said fourth solution comprises a decreased concentration of cryoprotectant relative to said third solution, said third solution comprises a decreased concentration of cryoprotectant relative to said second solution, and said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.

8. The method for establishing undifferentiated human embryonic stem cells of claim 5, further comprising a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant; wherein said third solution comprises about 0.1-2 vol % glycerol, said second solution comprises about 2-4 vol % glycerol, and said first solution comprises about 4-6 vol % glycerol.

9. The method for establishing undifferentiated human embryonic stem cells of claim 5, wherein at least one of said treating steps is carried out for about 4-6 minutes.

10. The method for establishing undifferentiated human embryonic stem
5 cells of claim 5, wherein said first solution and said second solution each comprise about 15-25% human follicular fluid.

11. The method for establishing undifferentiated human embryonic stem cells of claim 1, further comprising a step of removing trophectoderm from said embryo using anti-human lymphocyte antibody.

10 12. The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said portion of said embryo comprises the inner cell mass.

13. An undifferentiated human embryonic stem cell culture formed using the method of any one of claims 1 to 12.

15 14. A method for establishing undifferentiated human embryonic stem cells comprising the steps of:

(a) obtaining a population of cryogenically stored human embryos, wherein said population of embryos consists of embryos in the blastocyst phase;

(b) thawing one or more of said embryos; and

20 (c) culturing at least a portion of each of said one or more thawed embryos on a medium capable of sustaining undifferentiated embryonic stem cells; whereby undifferentiated human embryonic stem cells are established.

~~15. A method for thawing a cryopreserved human embryo comprising:~~

~~(a) a step of treating said cryopreserved human blastocyst embryo
25 with a first solution comprising human follicular fluid and cryoprotectant; and~~

(b) one or more subsequent steps of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant; wherein said second solution comprises a decreased concentration of cryoprotectant relative to said first solution; and wherein said embryo has been cryopreserved in a solution comprising human follicular fluid.

16. The method for treating a cryopreserved human embryo of claim 15, wherein said embryo is a blastocyst stage embryo.

17. The method for treating a cryopreserved human embryo of claim 15, wherein said cryoprotectant is selected from the group consisting of sucrose, glycerol and a combination of sucrose and glycerol.

18. The method for treating a cryopreserved human embryo of claim 15, wherein said subsequent steps consists of:

(c) treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant; and

(d) treating said cryopreserved human blastocyst embryo with a fourth solution comprising hFF and cryoprotectant; wherein said fourth solution comprises a decreased concentration of cryoprotectant relative to said third solution, and said third solution comprises a decreased concentration of cryoprotectant relative to said second solution.

19. The method for treating a cryopreserved human embryo of claim 15, further comprising a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant; wherein said third solution comprises about 0.1-2 vol % glycerol, said second solution comprises about 2-4 vol % glycerol, and said first solution comprises about 4-6 vol % glycerol.

20. The method for treating a cryopreserved human embryo of claim 15, wherein at least one of said treating steps is carried out for about 4-6 minutes.

21. The method for treating a cryopreserved human embryo of claim 15, wherein said first solution and said second solution each comprise about 15-
5 25% hFF.

22. A method for isolating the inner cell mass of a human blastocyst embryo comprising the step of treating said embryo with an anti-human lymphocyte antibody.

23. The method for isolating the inner cell mass of a human blastocyst
10 embryo of claim 22, wherein said embryo has been cryopreserved and thawed.

24. A method for establishing undifferentiated human embryonic stem cells comprising culturing at least a portion of the inner cell mass obtained by the method of claim 22 or claim 23 on a medium capable of sustaining undifferentiated embryonic stem cells; whereby undifferentiated human
15 embryonic stem cells are established.

25. An undifferentiated human embryonic stem cell culture formed using the method of claim 24.

26. An isolated inner cell mass of a human blastocyst formed using the method of claim 22 or claim 23.

20 27. A method of differentiating human embryonic stem cells comprising the steps of:

(a) thawing a cryopreserved human blastocyst embryo;

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(b) culturing at least a portion of said embryo on a medium capable of sustaining undifferentiated embryonic stem cells, whereby undifferentiated human embryonic stem cells are established; and

(c) differentiating said embryonic stem cells in a culture medium
5 comprising basic culture components and at least one growth factor.

28. The method for differentiating human embryonic stem cells of claim 27, wherein said differentiated cells are cardiac myocytes.

29. The method for differentiating human embryonic stem cells of claim 27, wherein said differentiated cells are muscle cells.

10 30. The method for differentiating human embryonic stem cells of claim 27, wherein said differentiated cells are nerve cells.

31. A differentiated cell prepared by the method of any one of claims
27 to 30.

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